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DOI:

[10.1016/j.ijpharm.2017.03.074](https://doi.org/10.1016/j.ijpharm.2017.03.074)

Document Version

Peer reviewed version

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Citation for published version (APA):

Hsieh, C-M., Warisnoicharoen, W., Patel, R. K., Kianfar, F., & Lawrence, M. J. (2017). Oil-in-water microemulsions stabilized by 3-(N,N- dimethylalkylammonio)propanesulfonate surfactants of varying alkyl chain length: solubilisation of testos-terone propionate. INTERNATIONAL JOURNAL OF PHARMACEUTICS. <https://doi.org/10.1016/j.ijpharm.2017.03.074>

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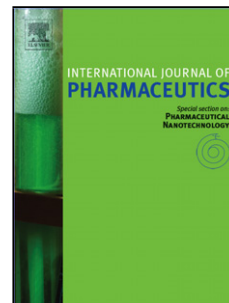
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Title: Oil-in-water microemulsions stabilized by 3-(N,N-dimethylalkylammonio)propanesulfonate surfactants of varying alkyl chain length: solubilisation of testos-terone propionate

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PII: S0378-5173(17)30261-2
DOI: <http://dx.doi.org/doi:10.1016/j.ijpharm.2017.03.074>
Reference: IJP 16546

To appear in: *International Journal of Pharmaceutics*

Received date: 15-11-2016
Revised date: 1-3-2017
Accepted date: 27-3-2017

Please cite this article as: Hsieh, Chien-Ming, Warisnoicharoen, Warangkana, Patel, Raju K., Kianfar, Farnoosh, Lawrence, M. Jayne, Oil-in-water microemulsions stabilized by 3-(N,N-dimethylalkylammonio)propanesulfonate surfactants of varying alkyl chain length: solubilisation of testos-terone propionate. *International Journal of Pharmaceutics* <http://dx.doi.org/10.1016/j.ijpharm.2017.03.074>

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Oil-in-water microemulsions stabilized by 3-(*N,N*-dimethylalkylammonio)propanesulfonate surfactants of varying alkyl chain length: solubilisation of testosterone propionate

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Keywords: 3-(*N,N*-dimethylalkylammonio)propanesulfonate; oil-in-water microemulsions; TP, ethyl ester oils

ABSTRACT

Solubilisation of the poorly-water soluble drug, testosterone propionate, in co-surfactant-free, dilutable, oil-in-water microemulsions stabilized by zwitterionic surfactants of varying alkyl chain length, namely 3-(*N,N*-dimethyloctylammonio)propanesulfonate and 3-(*N,N*-dimethyldodecylammonio)propanesulfonate and containing one of four ethyl ester oils, has been investigated. Both 3-(*N,N*-dimethyloctylammonio)propanesulfonate and 3-(*N,N*-dimethyldodecylammonio)propanesulfonate-stabilized microemulsions containing two short chain length oils, ethyl butyrate and ethyl caprylate, while only 3-(*N,N*-dimethyldodecylammonio)propanesulfonate formed microemulsions incorporating the longer chain length oils, ethyl palmitate and ethyl oleate, albeit to a very much reduced extent. Significantly the microemulsions containing the short chain length oils, ethyl butyrate and ethyl caprylate solubilised more testosterone propionate than the corresponding micelles. However, an inverse correlation existed between testosterone propionate solubility in the bulk oil and solubilisation in the microemulsions, in that ethyl caprylate containing microemulsions solubilised more testosterone propionate than those containing an equivalent amount of ethyl butyrate, despite the drug being more soluble in ethyl butyrate. These results suggest that drug solubility in bulk oil is a poor indicator of drug solubility in microemulsions containing that oil, and whether or not the addition of oil improves drug solubility is dependent upon on how it is incorporated within the microemulsion. The longer the chain length of the oil, the more likely the oil is to form a core in the microemulsion droplet, resulting in an additional locus of drug solubilisation and the possibility of an enhanced solubilisation capacity.

NOTE

Oil-in-water (o/w) microemulsions are attractive vehicles for the solubilisation of water-insoluble and sparingly water-soluble compounds for pharmaceutical application (Lawrence and Rees, 2012). Previous research has shown that the apparent aqueous solubility of potent, low dose drugs of intermediate lipophilicity can be significantly enhanced when solubilised in an o/w microemulsion as opposed to the corresponding micellar solution (Malcolmson and Lawrence 1993, Malcolmson et al, 1998). The reason proposed for this is the presence of a core of oil in the microemulsion droplet can act as an additional locus of drug solubilisation, assuming that the drug exhibits a reasonable level of solubility in the

oil (Malcolmson et al., 1998) and, that the oil is present in a ‘fluid’ state inside the microemulsion (Wasutrasawat et al., 2013). As shown by small angle neutron scattering studies, whether an oil forms a core in the microemulsion droplet is dependent upon both the amount of oil incorporated and the relative chain lengths of the oil and surfactant, with oils of comparable or longer chain length to the surfactant being more likely to form a core (Barlow et al., 2000; Hsieh, 2010; Wasutrasawat, 2014).

Little work has been performed to investigate the effect of surfactant head group on the solubilisation of poorly water soluble drugs within o/w microemulsions. To date our group has reported on the ability of o/w microemulsions, prepared using a polyoxyethylene surfactant to solubilise drug (Malcolmson and Lawrence 1993, Malcolmson et al., 1998). The present study reports on the ability of surfactants possessing a zwitterionic ammoniopropylsulfonate head group, and either a C8 or C12 hydrocarbon chain, namely 3-(*N,N*-dimethyloctylammonio)propanesulfonate and 3-(*N,N*-dimethyldodecylammonio)propanesulfonate, to form o/w microemulsions containing a range of ethyl ester oils and for these microemulsions to solubilise testosterone propionate (mean water solubility of 0.0009 ± 0.002 %w/w (Malcolmson et al., 1998); log *P* of 4.78 (Craig, 1990)). The results are compared with the level of drug solubilisation obtained in the corresponding micellar solutions. Surfactants containing an ammoniopropylsulfonate head group was selected for study as this class of surfactant have previously been reported to demonstrate a good solubilising capacity for both oil and drug (Hsieh, 2010; Saaka, 2016). In addition, zwitterionic ammoniopropylsulfonate surfactants exhibit a number of pharmaceutical advantages, including little sensitivity towards salt and pH over a physiologically relevant range (Hsieh, 2010; Myers 2006). The aim of the present study is to understand how best to formulate a microemulsion to achieve an increase in drug solubilisation.

Phase diagrams were constructed by preparing a large number of individual samples by simple mixing and stirring of the required amounts of oil (ethyl butyrate (EB), ethyl caprylate (EC), ethyl oleate (EO), ethyl palmitate (EP); Sigma chemical Co. Ltd, Dorset, UK), surfactant (3-(*N,N*-dimethyloctylammonio)propanesulfonate (DOAPS), 3-(*N,N*-dimethyldodecylammonio)propanesulfonate (DDAPS); Fluka Chemica Ltd., Derbyshire, UK) and water (double distilled water from a well-seasoned, all glass still). When EO and EP were used as oils, the samples were heated to 70 °C for 15 minutes to speed up the

microemulsification process, which took an extended time to occur at room temperature with these oils. When EB and EC were used as oils, microemulsification occurred almost immediately at room temperature. All the resulting samples were stored at 22 ± 2 °C in tightly-sealed glass containers. Samples that remained clear, non-birefringent, and fluid after one-month storage were classified as microemulsions. The level of drug (testosterone propionate (TP); Sigma chemical Co. Ltd, Dorset, UK) solubilisation at 22 ± 2 °C in DOAPS and DDAPS micelles and microemulsions was determined as described by Malcolmson and Lawrence (1993).

Figure 1 shows the ability of DOAPS and DDAPS to form o/w microemulsions with the four ethyl ester oils examined. All systems, with the exception of the DOAPS systems containing either EO or EP, formed microemulsions, albeit to greatly differing extents. As the oils all possess very similar densities, if the various oils were being incorporated into microemulsions formed by the same surfactant in the same way, then the area of existence would be expected to be comparable, which clearly is not the case here. This result provides *indirect* evidence for different oils being preferentially located at different sites within the microemulsion droplets. In some systems (e.g. those containing DOAPS and EC), microemulsion formation did not occur until 35 %w/w surfactant was present. The maximum amount of oil solubilisation occurred at high surfactant concentrations of ~ 40 -45 %w/w. These surfactant concentrations were far higher than those recorded for microemulsions containing the same oils but stabilized using dimethyldodecylamine-*N*-oxide, polyoxyethylene-10-dodecyl ether and the longer hydrophobic chain surfactant, polyoxyethylene-10-oleyl ether, where solubilisation occurred over a much narrower range of surfactant concentrations, with maximum oil solubilisation typically occurring at ~ 20 -25 %w/w (Warisnoicharoen et al., 2000). These differences are likely to be a consequence of the surfactant concentration range over which the corresponding micelles form.

Both DOAPS and DDPAS solubilised the shortest chain oil, EB, to the greatest extent and DDAPS, the longest chain oil, EO, to the least extent. The inverse correlation between oil incorporation and molecular volume has previously been reported for a number of other C12 surfactants including polyoxyethylene and amine-*N*-oxide surfactants (Malcolmson and Lawrence, 1993; Warisnoicharoen et al., 2000). Significantly, in the present study, although it was not possible to prepare o/w microemulsions at low surfactant concentrations

(i.e. < 5 %w/w) using the methodology described, it was possible to produce stable DOAPS and DDAPS microemulsions upon dilution with water. An important property if such microemulsion vehicles are to be used for drug delivery.

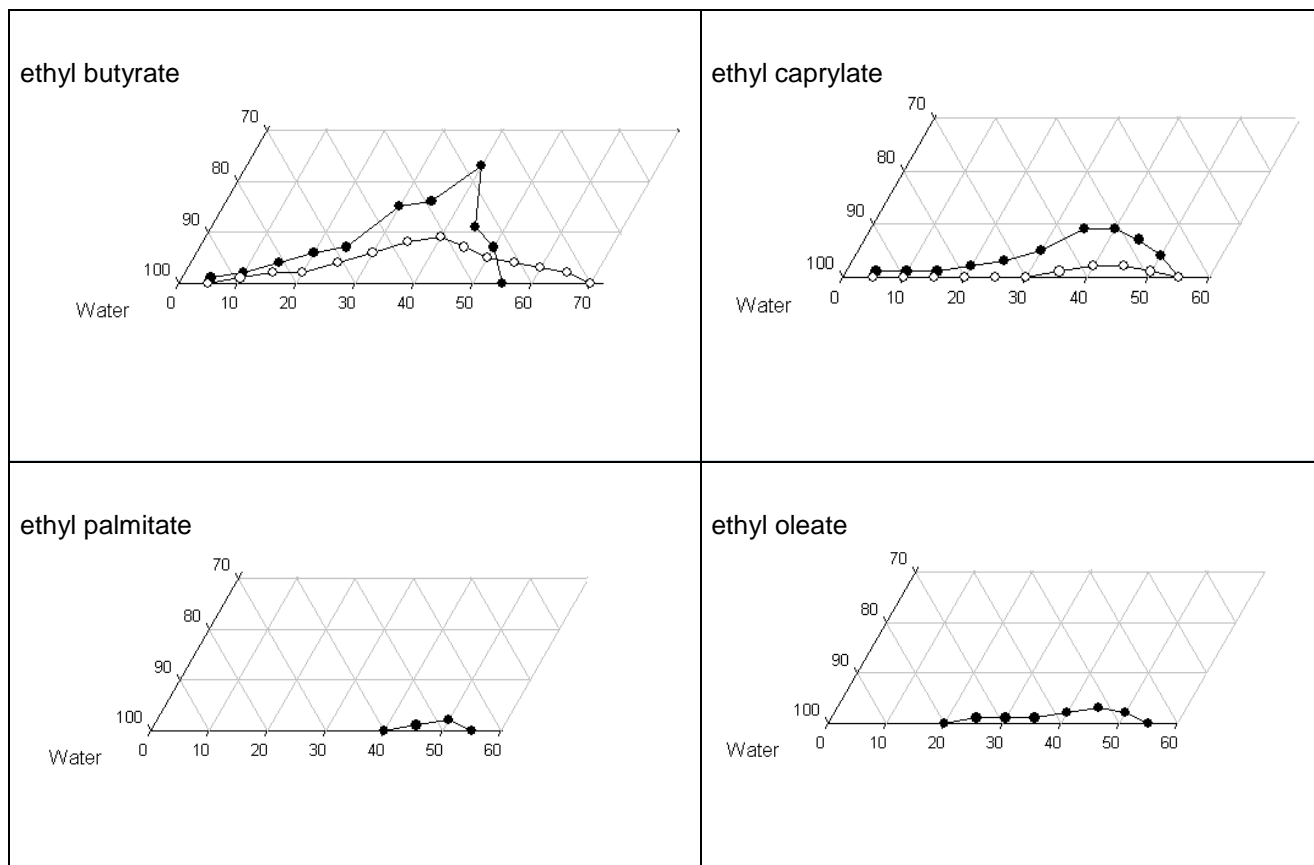


Figure 1: Partial triangular phase diagrams for o/w microemulsions formulated at 22 ± 2 °C with 3-(*N,N*-dimethyloctylammonio)propanesulfonate (○) or 3-(*N,N*-dimethyldodecylammonio)propanesulfonate (●) and containing either ethyl butyrate, ethyl caprylate, ethyl palmitate or ethyl oleate. Microemulsions are formed within the area between the abscissa and the boundary demarked by the line drawn on the phase diagram. On the abscissa, % w/w surfactant concentration increases from left to right while on the ordinate % w/w oil concentration increases from bottom to top. The apex of the triangle shown represents 100% w/w water.

As anticipated the ability of the DOAPS and DDAPS micelles to solubilise TP increased with surfactant concentration over the range 5-35 %w/w (Table 1). The solubilisation capacity of the DDAPS micelles (~ 0.06 g/g), however, was approximately three times higher than that observed for the DOAPS micelles (~ 0.02 g/g) and is most likely the result of DDAPS's ability to form larger sized micelles (Navarrette and Serrano, 1983; le Maire et al, 2000).

Table 1: Solubilisation of testosterone propionate (TP) into 3-(*N,N*-dimethyloctylammonio)propanesulfonate (DOAPS) and 3-(*N,N*-dimethyldodecylammonio)propanesulfonate (DDAPS) micelles at 22±2 °C.

Surfactant concentration (%w/w)	%w/v drug solubilized ±SD in micellar solutions	
	DOAPS	DDAPS
5	0.155±0.033	0.787±0.058
10	0.230±0.033	0.852±0.029
15	0.373±0.004	0.863±0.021
20	0.466±0.006	1.134±0.013
25	0.509±0.008	1.515±0.106
30	0.571±0.022	1.740±0.040
35	0.734±0.018	2.044±0.026

Solubilisation data obtained for TP can be converted from %w/v to mg mL⁻¹ by multiplying by 10.

Due to the high concentration of surfactant needed to form DOAPS and DDAPS microemulsions, TP solubilisation was determined at surfactant concentrations of 35 %w/w (Table 2). Regardless of the amount of oil added, TP solubilisation was greater in *all* microemulsions studied compared to the equivalent micellar solution. Furthermore, EC containing DDAPS microemulsions solubilized TP to a greater extent than those containing EB at the same surfactant concentration (Table 2). TP solubilisation in EP and EO containing microemulsions was not investigated due to the poor ability of both DOAPS and DDAPS to form microemulsions containing these oils. Similarly TP solubilisation in DOAPS microemulsions containing EC was not measured due to the extremely poor extent of microemulsion formation observed in this system.

Table 2: Solubilisation of testosterone propionate (TP) into 3-(*N,N*-dimethyloctylammonio)propanesulfonate (DOAPS) and 3-(*N,N*-dimethyldodecylammonio)propanesulfonate (DDAPS) micelles and microemulsions containing 35% w/w surfactant at 22±2 °C.

Amount of oil (%w/w)	%w/v TP incorporated ± SD in DOAPS microemulsions		%w/v TP solubilised ± SD in DDAPS microemulsions			
	Ethyl butyrate		Ethyl butyrate		Ethyl caprylate	
	Actual	Predicted	Actual	Predicted	Actual	Predicted
2	0.997±0.049*	1.11	2.160±0.009	2.42	2.676±0.024*	2.29
4	1.080±0.003*	1.48	2.429±0.093*	2.79	2.668±0.011*	2.53
6	1.291±0.020*	1.85	2.682±0.054*	3.16	3.253±0.040*	2.77
8	1.384±0.060*	2.23	2.820±0.170*	3.54	3.496±0.002*	3.02
10	N/F		3.166±0.129*	3.91	N/F	-
12	N/F		4.071±0.038*	4.28	N/F	-
14	N/F		4.149±0.029*	4.65	N/F	-
16	N/F		4.359±0.115*	5.03	N/F	-
micelles	0.734±0.018		2.044±0.026		2.044±0.026	

Asterisk indicates a significant difference ($p \leq 0.01$) between uptake into microemulsions and micelles at equivalent surfactant concentrations ($n = 3$).

N/F = microemulsions not formed.

No results obtained for EC containing DOAPS microemulsions due to very small microemulsion region obtained.

Solubilisation data obtained for TP can be converted from %w/v to mg mL⁻¹ by multiplying by 10.

Table 2 also shows the solubility of TP predicted in microemulsions assuming that the final solubility is determined solely by the drug's solubility in the oil (EB ~ 18.6 %w/w; EC ~ 12.2 %w/w; Malcolmson et al., 1998), assuming that the oil forms a distinct core in the microemulsion droplet. Although the predicted solubility is greater in the EB-containing DDAPS microemulsions, the measured solubility is greater in the EC containing microemulsions, with the actual solubility achieved in the EB and EC containing systems being ~ 86% and 114%, respectively of that predicted. This observation is undoubtedly a consequence of the different ways the two oils are incorporated into the DDAPS microemulsions with EC most likely forming a bigger core and therefore a larger additional locus of solubilisation. Interestingly, although the amount of TP solubilized increased with EB content in the DOAPS microemulsions, solubility decreased from ~ 89% of that predicted to ~ 62% as EB

content increased from 2 to 8 wt%. Correspondingly, there was no clear correlation between TP solubility in bulk oil and in the microemulsion.

The present study suggests the ability of o/w microemulsion stabilized by the two ammoniopropanesulfonate surfactants to increase the solubilisation of TP depends upon the alkyl chain length of the surfactant, the amount of surfactant in the microemulsion, and the amount and nature of the incorporated oil – with the nature of oil incorporation plays a pivotal role in for drug solubilisation.

References

- Barlow, D.J., Lawrence, M.J., Zuberi, T., Zuberi, S. and Heenan, R.K., Small angle neutron scattering studies on the nature of the incorporation of polar oils into aggregates of *N,N*-dimethyldodecylamine-*N*-oxide. *Langmuir* 16 (2000) 10398-10403.
- Craig, P.N., Drug Compendium. In *Comprehensive Medicinal Chemistry*. Vol.6: Cumulative Subject Index and Drug Compendium; Hansch, C., Sammes, P.G.: Taylor, J. B., Eds. Pergamon Press: New York, 1990; pp.237-991.
- Hsieh, C.-M., (2010) Investigations of pharmaceutical oil-in-water (o/w) microemulsions as drug delivery systems. PhD Thesis, University of London.
- Lawrence, M.J. and Rees, G.D., Microemulsion-based media as novel drug delivery system. *Adv. Drug Del. Rev.*, 64 (2012) 175-193.
- Le Maire, M., Champeil, P. and Møller J.V. Interaction of membrane proteins and lipids with solubilizing detergent. *Biochim. Biophys. Acta* 1508 (2000) 86-111.
- Malcolmson, C. and Lawrence, M.J., A comparison of the incorporation of steroids into nonionic micellar and microemulsion systems. *J. Pharm. Pharmacol.* 45 (1993) 141-143.
- Malcolmson, C., Satra, C., Kantaria, S., Sidhu, A. and Lawrence, M.J., Effect of oil on the level of solubilization of TP into nonionic oil-in-water microemulsions. *J. Pharm. Sci.*, 87 (1998) 109-116.
- Myers, D., *Surfactant Science and Technology* Third Edition Wiley-Interscience. John Wiley and Sons, Inc: New Jersey, 2006; pp 76.
- Navarrete, R. and Serrano, R., Solubilisation of yeast plasma membranes and mitochondria by different types of non-denaturing detergents. *Biochim. Biophys. Acta* 728 (1983) 403-408.

Saaka, Y., (2016) Effect of surfactant headgroup on the micellar solubilisation of poorly water soluble drugs. PhD Thesis, King's College London.

Warisnoicharoen, W., Lansley, A.B. and Lawrence, M.J., Nonionic oil-in-water microemulsions: the effect of oil type on phase behaviour. *Int. J. Pharm.*, 198 (2000) 7-27.

Wasutrasawat, P., Al-Obaidi, H., Gaisford, S., Lawrence, M.J., Warisnoicharoen, W., Drug solubilisation in lipid nanoparticles containing high melting point triglycerides. *Eur. J. Pharm. Biopharm.*, 85 (2013) 365-371.

Wasutrasawat, P., (2012) Formation of physicochemical properties of non-ionic oil-in-water nanoemulsions containing liquid and solid triglycerides. PhD Thesis, Chulalongkorn University.